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ABSTRACT

Intended to supplement a regular program, this pamphlet provides background information, related activities, and suggestions for other activities on the subject of nitrate as a water pollutant. Two activities related to plant nutrient pollution, nitrate filtration and measuring mitrate used by plants, are explained in detail, outlining objectives, materials required, procedure, and questions. The third part of this Science Study Aid describes a technique for measuring the amount of nitrate in water, and the amount necessary to carry out the two activities. A vocabulary list and bibliography are included together with diagrams and space for taking notes. (BL)

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# NITRATE WATER ACTIVITIES

U.S. DEPARTMENT OF AGRICULTURE — Science Study Aid No. 4

## TEACHER'S INTRODUCTION

This Science Study Aid provides background information, related activities, and suggestions for other activities on the subject of nitrate as a water pollutant. With this background, the student and teacher will be able to develop followup activities relevant to the situation in their community.

It would be helpful if the student has some knowledge of how water leaches materials from the soil. The student should also be familiar with titration techniques and the nitrification and denitrification processes. Several references and suggested activities relating to the nitrate-water problem are listed in this Science Study Aid. They offer relevant background information as well as opportunities for students interested in additional study.

### Objectives

Completion of these activities should enable the student to:

1. Run an accurate nitrate analysis of a water sample.
2. Explain the rate of nitrate use by plants in relation to their life cycle.
3. Explain how filtration reduces the nitrate content of water.
4. Write additional activities or adaptations of the material.

### Suggested Use

You may wish to reproduce the following material for distribution to students. A general background statement begins on page 2. Two activities follow. One is a method for removing nitrate from water, and the other is a method for measuring nitrate used by plants in different stages of growth. The third part of this Science Study Aid describes a technique for measuring the amount of nitrate in water. This measuring technique is necessary to carry out the two activities.

*Nitrate Water Activities* was developed by Joan Valieant and Jerry L. Southland. Miss Valieant is an environmental curriculum development specialist with the Montgomery County school system in Maryland. Mr. Southland is a junior high school teacher in Grand Rapids, Michigan. They worked closely with soil scientists at the Agricultural Research Center at Beltsville, Maryland.

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## BACKGROUND INFORMATION

Providing adequate water to meet the increasing demands of our society is becoming an enormous problem. The Nation's research community is responding by focusing more and more of its attention on ways to conserve and recycle water.

The United States has an average annual rainfall of 30 inches. About 21 inches of this returns to the atmosphere as transpiration from plants or evaporation from soil and water. The remaining 9 inches of rainfall is natural runoff water. Increased withdrawals from this relatively constant water supply will require more recycling of water for reuse.

Some experts predict that multiple reuse of water will be necessary by 1980, when withdrawals of water will equal or exceed the supply. In water-short areas, such as exist in the Southwest, waste-water reuse has already arrived.

What we are talking about then is reuse of "used" or "polluted" water that may have to be reconditioned. Eight general categories of pollutants are: common sewage and other oxygen-demanding wastes; disease-causing agents; plant nutrients; synthetic organic chemicals; inorganic chemicals and other mineral substances; sediment; radioactive substances; and heat.

The accompanying science activities are related to plant-nutrient pollution. Plant nutrients support and stimulate the food chain of aquatic life, such as algae and water weeds. Nitrogen and phosphorus are the two chief nutrients present in small amounts in natural water; much larger amounts are contributed by sewage, certain industrial wastes, and drainage from fertilized lands.

When the concentration of nutrient minerals in lakes and rivers becomes too high, growth of water plants usually becomes excessive. The water may then become undesirable for domestic and recreational uses.

Cities, livestock operations, and food processing plants all concentrate food, feed, and other agricultural products. Once digested or processed, these products return to our environment as sewage, manure, or processing wastes. Biological waste-treatment processes do not remove the nutrients—in fact, they convert the organic forms of these substances into mineral form, making them more usable by plant life.

Although sewage provides the major source of plant nutrients added to our water, chemicals in runoff from agricultural lands have also been blamed as one of the prime contributors to over-enrichment (eutrophication) of surface waters.

More information is needed for evaluating the amounts and sources of agriculture's contribution to this eutrophication problem. Fertilizers, for example, are now being applied at higher rates than formerly because they are cheaper and because of the economic need to get the largest crop returns per acre. This increased use of fertilizers could increase concentrations of chemicals in agricultural runoff.

Because nitrogen is highly soluble, many people are quick to associate statistics on the rapid expansion of fertilizer use with suspected increases in water pollution from nitrate; however, the behavior of nitrogen in soil is highly complex. In addition to the nitrogen added to the soil in the form of fertilizers, there are at least five other sources to consider. They are:

1. The organic matter in the soil and the rate at which soil organisms convert it to nitrate.
2. The atmospheric nitrogen that is fixed either symbiotically or nonsymbiotically by microbial action in soil.
3. The nitrogen involved in crop utilization and leaching.
4. The nitrogen assimilated by microorganisms.
5. The nitrogen returned to the atmosphere.

In the dynamic soil-plant system, these processes take place simultaneously. Thus, when

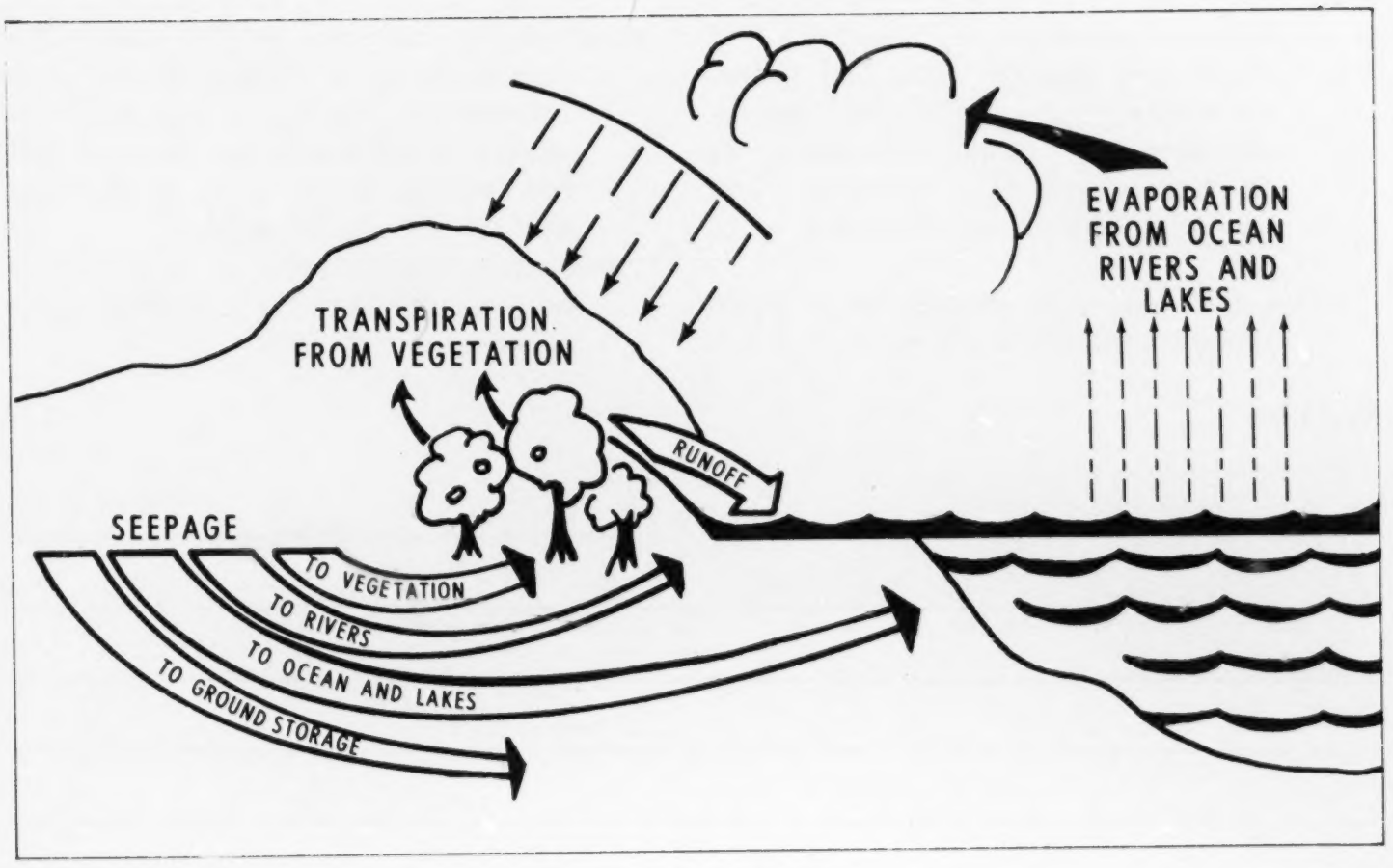


Figure 1.—Hydrologic cycle.

nitrate is found in water, it is difficult to determine if fertilizers are the source.

Furthermore, the overall nutrient requirements of algae and various aquatic plants are only partially understood. Because of the present lack of knowledge, we cannot state categorically that above-normal levels of nitrogen and phosphorus in waters will result, inevitably, in algae blooms.

A more obvious and direct danger is a high-nitrate content in drinking water. High-nitrate water, when consumed by babies, can change part of the hemoglobin to methemoglobin, a blood component that causes oxygen starvation. This disease, methemoglobinemia, is relatively rare in babies, and adults are seldom affected. The Public Health Service has set 10 parts per million as the maximum desirable limit for nitrate-nitrogen in drinking water.

## VOCABULARY

anaerobic—able to grow in an oxygen-free atmosphere, deriving oxygen from nitrate compounds.

denitrification—the reduction of nitrate to nitrite, with the formation of ammonia and free nitrogen, as in nitrate reduction in soil by soil organisms, particularly anaerobic organisms under certain conditions.

effluent—liquid discharged as waste.

eutrophication—the process of becoming over-enriched in plant nutrients, either as a natural phase in the maturation of a body of water or artifically, as by fertilization.

leaching—the process of separating the soluble components from a material by percolation.

nitrification—oxidation by bacteria of ammonium salts to nitrite and further oxidation of nitrite to nitrate wherever the proper conditions of temperature, air, moisture, and alkalinity allow the nitrobacteria to act.

percolation—the slow passage of a liquid through a filtering medium.

titration—the volumetric determination of a constituent in a known volume of a solution by the slow addition of a reacting solution of known strength until the reaction is completed, as indicated by a color change (indicators).

transpiration—the emission or exhalation of watery vapor from the surfaces of leaves or other parts of plants.

NOTES:

## ACTIVITY I: Nitrate Filtration

The following activity is based on an ARS pilot project in Phoenix, Arizona. In this project, the effluent from the city of Phoenix passes through a conventional sewage plant and is admitted into shallow basins (recharge basins) using a plant-soil filter system covering 1,000 acres. The effluent is purified as it percolates through the soil. When it reaches the ground water table, it moves laterally, as ground water, to a well or other collection facility.

Under the best conditions, one acre of this filter system could process 300 acre-feet or more of secondary sewage effluent per year. This amounts to approximately 100 billion gallons per year, or 270 million gallons per day. One of the major advantages of this process is its low cost. It is one-tenth the cost of chemical revitalization.

The following activity is a microversion of the project in Phoenix. You will learn how to construct your own soil-filter system to filter and purify water that contains nitrates.

For the system to work effectively, the soil must be saturated with the water passing through it. As the soil becomes saturated, anaerobic conditions result. Under these conditions, anaerobic bacteria use nitrate as a source of oxygen. The microorganisms metabolize the oxygen from the nitrate, causing the release of free nitrogen into the atmosphere. This process is called denitrification.

The technique explained on page 10 can be used to chemically analyze the water for nitrate content before and after filtration. Other methods of chemical and biological analysis can also be used.

NOTE: The material you use for filtering will vary in its capacity to remove nitrate. It will also vary in permeability. Consider these factors because they will affect your results. You can develop many variations of this activity by varying the length of the filter, the type of filtering material, etc.

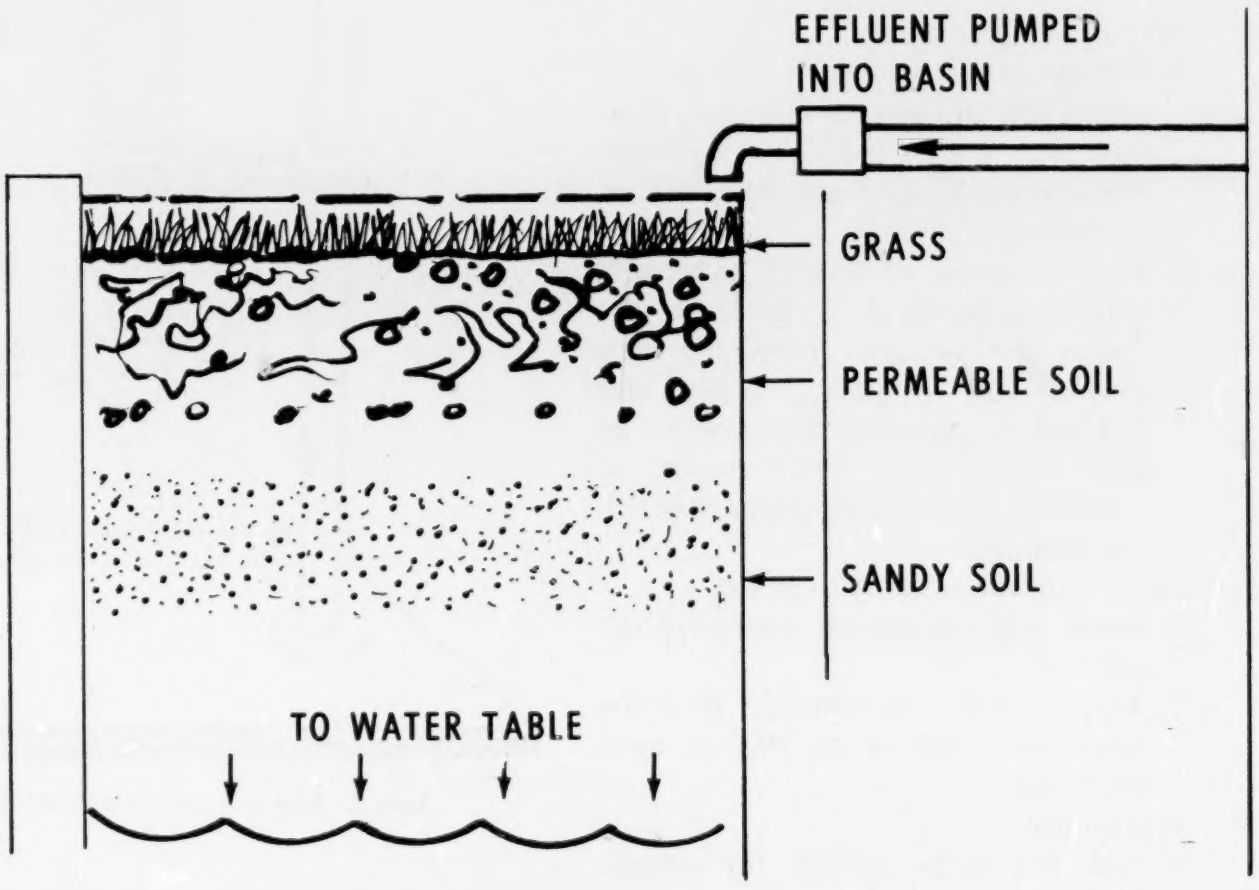


Figure 2.—Flushing Meadows Pilot Project.

*Objective:*

To measure nitrate present in water and to remove nitrate from water by denitrification during filtration.

*Materials:*

Two (or more, depending on number of samples of test water) plastic or glass columns approximately 3 feet long and 1-1/2 inches wide. The Earth Science Curriculum Project (ESCP) equipment may be used.
Distilled water.
Gravel, sand, soil.
Glass tubing—5 mm. outside diameter.
One-holed rubber stoppers—size # 8.
Erlenmeyer flasks 350 ml. (suggested size).
Beakers 400 ml. (suggested size).
Ring stands to support columns.
Test tube clamps to support columns.
NOTE: You will also need materials for the nitrate test, see page 10.

*Procedure:*

A. Setting up apparatus.
   1. Prepare two columns: one for the actual test and one for a control. Feed the glass tubing into the one-holed stoppers and fit each one into the base of each glass column.
   2. Feed a piece of cotton into each column, placing it at the base just above the stopper. Then fill each column with 1/3 gravel, 1/3 sand, and 1/3 soil, in that order from bottom to top. Leach out any nitrate, if necessary, by running water through the columns.

B. Water samples containing nitrate.
   1. Water that has leached well-fertilized soil.
   2. Any water containing decaying substances, such as raw sewage, dead leaves, etc.

C. Nitrate test.
   1. Test the water sample for nitrate. Refer to the nitrate test on page 10.

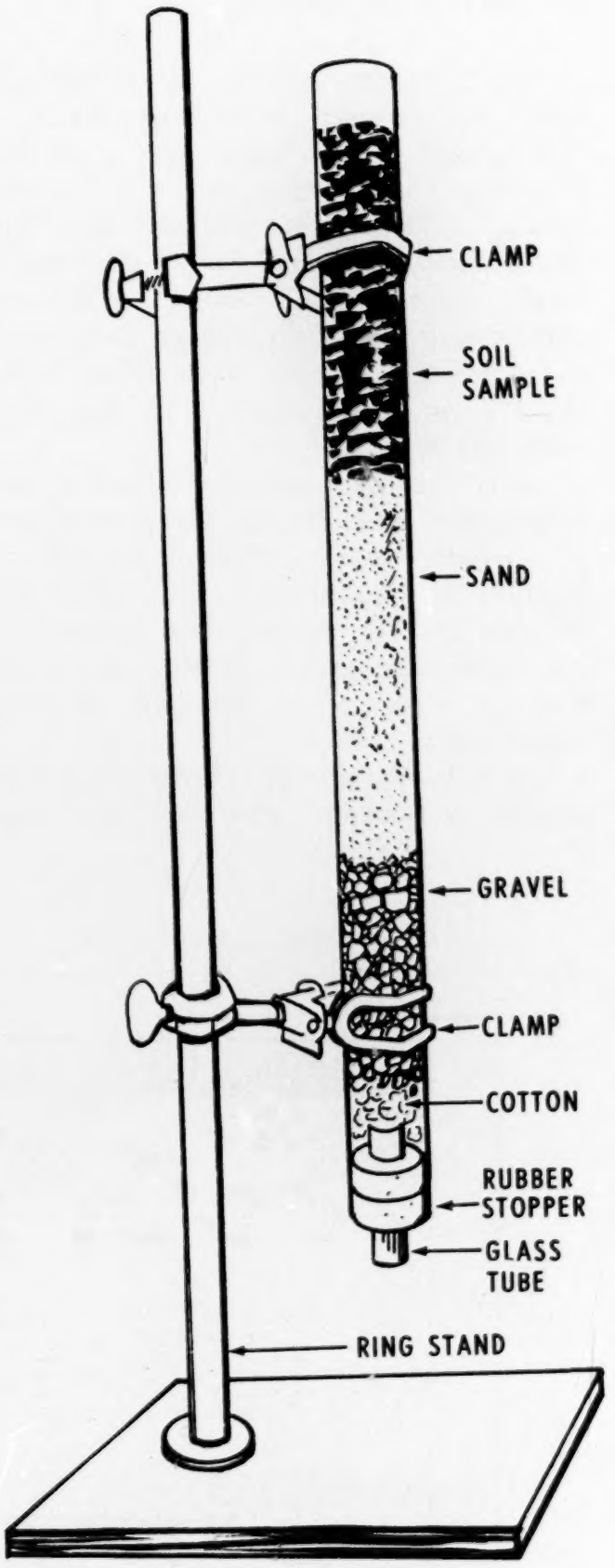


**Figure 3.—Apparatus for nitrate filtration.**

2. Test the distilled water or tap water to be used as a control sample.

D. Filtration Process.

1. Pour into one glass column as much test water as it will hold; continue pouring the water (in order to saturate the column) until you have obtained the desired amount of filtrate. (Only 4 ml. are needed per test.)

2. Into the second glass column, pour distilled water or tap water and follow the same procedures as in #1 above.

E. Repeat the nitrate test on filtered samples.

*Questions and Suggestions:*

1. How effective do you think this technique is in removing nitrates?
2. What causes the nitrates to be removed by the soil?
3. What other pollutants might be removed by this technique? Try some other standard qualitative tests for chemicals, outlined in any standard high school chemistry book.
4. Devise a technique for sampling the water for microorganisms before and after filtration.
5. What are the differences between primary, secondary, and tertiary stages in the purification of water?

NOTES:

## ACTIVITY II: Measuring Nitrate Used by Plants

In this activity, you will measure the amount of nitrate used by various plants. By using plants of different ages, you will discover at what stages of development plants require the maximum amount of nitrate.

The plants are grown in a nutrient nitrate solution, because it allows a direct quantitative measurement of nitrate using the Conway Method (see nitrate test on page 10). This method can be used to measure the ammonium ($NH_4$) content as well as the nitrate ($NO_3$) content.

*Objective:*

To measure and determine the amount of nitrate used by plants at different stages of development.

*Materials:*

Marking pencil.

Three corks of the size to fit the mouth of the jars you use. In the center of each cork, make a hole large enough to fit the stems of three plants.

Three jars. (More will be needed if you wish to test duplicate samples.)

Plant nutrient solution containing nitrate. Any commercial liquid fertilizer can be used. **Suggestion:** Follow the directions on the container when preparing the nutrient solution.

Three corn plants at each age: 1, 2, and 3 weeks. Other plants may be substituted for the corn plants.

NOTE: You will also need materials for the nitrate test, see page 10.

*Procedure:*

1. Fill each of the three jars to a level that covers the roots. Use the same amount of nitrate nutrient solution in each jar.

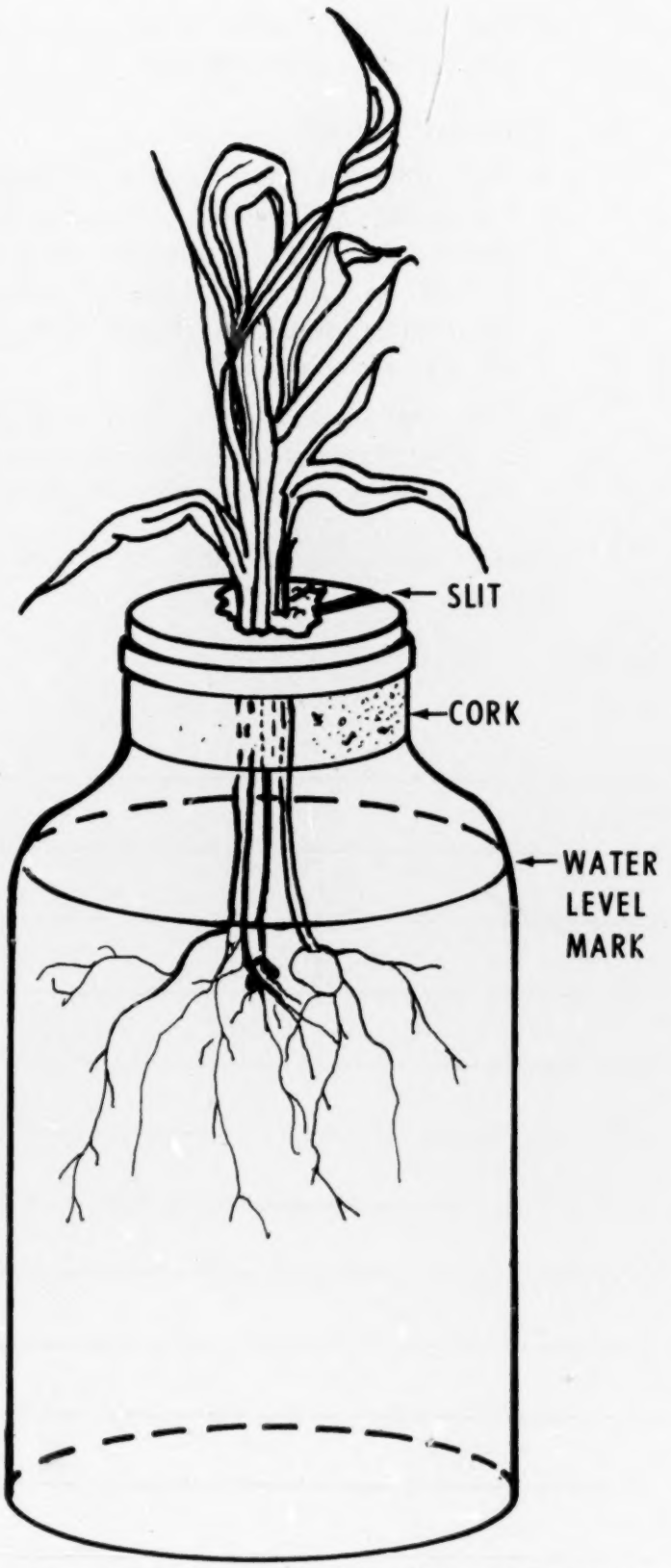


**Figure 4.—Apparatus for measuring nitrate used by plants.**

2. Test the $NO_3$ and $NH_4$ content of the solution using the Conway Method. Record the amounts detected.
3. Indicate the level of solution on each jar with a marker, and label the jars.
4. Insert the corn plants into each jar and through each cork. Use 3 plants of the same age for each jar. For easier insertion of plants into the center hole of the cork, make a slit in the cork from the edge to the center hole. After plants have been inserted, stuff cotton between the stems and cork to anchor the plants.
5. Aerate the nitrate nutrient solution 1 minute twice a day. Use an air line, water aspirator, or hold plants out of the solution and stir vigorously.
6. At the end of one day (24 hours), fill the containers up to the original marks with distilled water. This is necessary because there will be a loss of solution due to transpiration from plants. The volume of the liquid must remain constant to provide accurate quantitative results for the second nitrate analysis.
7. Test the solution for amounts of $NO_3$ and $NH_4$ present. The amounts lost will represent those used by the plants. The activity can be terminated at the end of one day or you can run the nitrate test again at the end of two or three days, depending on the time available.

*Questions:*

1. Is there a particular stage in the development of corn plants when the plant requires higher concentrations of nitrate? Explain.
2. Does the amount of water used by the plant in any way affect the amount of nitrate utilized? Explain.
3. How can the accumulation of nitrate in water sources be controlled by the grower if he has knowledge of the plant's need for nitrate?

*Supplementary Activities:*

1. It is suggested that the student try testing other kinds of plants. A comparison might be drawn between those plants which support nitrogen-fixing bacteria, such as bean plants, and those which depend instead on available nitrate in the soil.
2. Use two different concentrations of nitrate solution to determine whether the concentration of the nitrate present has any effect on the amount the plant utilizes.
3. Leave samples of distilled water and water with nitrate added in an exposed area to test for algae growth.

NOTES:

**FOR ACTIVITIES I & II:** A Nitrate Test (Conway Method)

There are many sophisticated techniques for determining the amount of nitrate in water. The Conway Method, described here, is a relatively simple procedure for a quantitative analysis for nitrate and ammonia.

(Note: If you do not have the necessary equipment for the Conway test, a less precise but simpler nitrate test can be made with a soil testing kit. These are available from most scientific supply companies.)

In this method, Devarda's alloy in an alkaline solution produces hydrogen, which transforms all nitrate in the sample water into ammonium ($NO_3^- + 5H_2 \rightarrow NH_4^+ + 3H_2O$). This diffuses as a gas, ammonia, to react with an indicator solution. When titrating this solution back to its original color with a known strength sulfuric acid, you can determine by the amount of acid used how much nitrate was in the original water sample.

*Materials:*

Twelve, 68 millimeter, Obrink-Conway microdiffusion dishes and covers. Available from Bel-Art Products, Inc., Pequannock, N.J. 07440; Fisher Scientific Co. (local office); or Matheson Scientific Co. (local office).
One microburette with a 0.2 ml. capacity, calibrated to 0.2 microliters.
One 4 ml. pipette.
One 10 ml. pipette.
Several beakers.
Devarda's alloy (reagent grade).
$H_3BO_3$ (boric acid) reagent grade.
Methyl red.
Bromocresol green.
95% ethanol.
0.1 normal NaOH (sodium hydroxide).
0.02 normal $H_2SO_4$ (sulfuric acid).
40% $K_2CO_3$ (potassium carbonate).
Tergitol (for use in boric acid solution).
Wash bottle.

*Preparation of Materials:*

A. Mixing indicators.
   1. In an agate mortar, grind 0.066 g. of methyl red, then add 2 ml. of 0.1 normal NaOH. Transfer to a 100 ml. volumetric flask and dilute to 70 ml. with 95% ethanol.
   2. In an agate mortar, grind 0.033 g. of bromocresol green, then add 1 ml. of 0.1 normal NaOH. Add this to the methyl red solution and fill the flask to volume with water. This mixture is 0.033% bromocresol green and 0.066% methyl red.

B. Preparing 1% boric acid solution with indicators.
   To a liter volumetric flask, add 10 g. of reagent grade $H_3BO_3$ crystals, 200 ml. of 95% ethanol, 700 ml. of water, 10 ml. of mixed indicator, and 0.25 ml. of tergitol. Stir until the $H_3BO_3$ is dissolved. Add water to the one-liter mark.

C. Preparing Devarda's alloy.
   In a mortar, grind to a fine powder approximately 50g. of this reagent-grade alloy. This alloy acts as the reducing agent to convert the nitrate to ammonium. Approximately 30 mg. are used in each Conway dish.

*Procedure:*

1. Pipette 1 ml. of boric acid indicator solution into the center ring of each of the four Conway dishes (number 1, 2, 3, 4). Make sure that none of the substances from one ring get into other rings of the diffusion unit.
2. Using a fine spatula, distribute fairly evenly approximately 30 mg. of Devarda's alloy into the second ring of Dish #1.
3. Pipette 4 ml. of the solution to be analyzed into the second ring with Devarda's alloy.

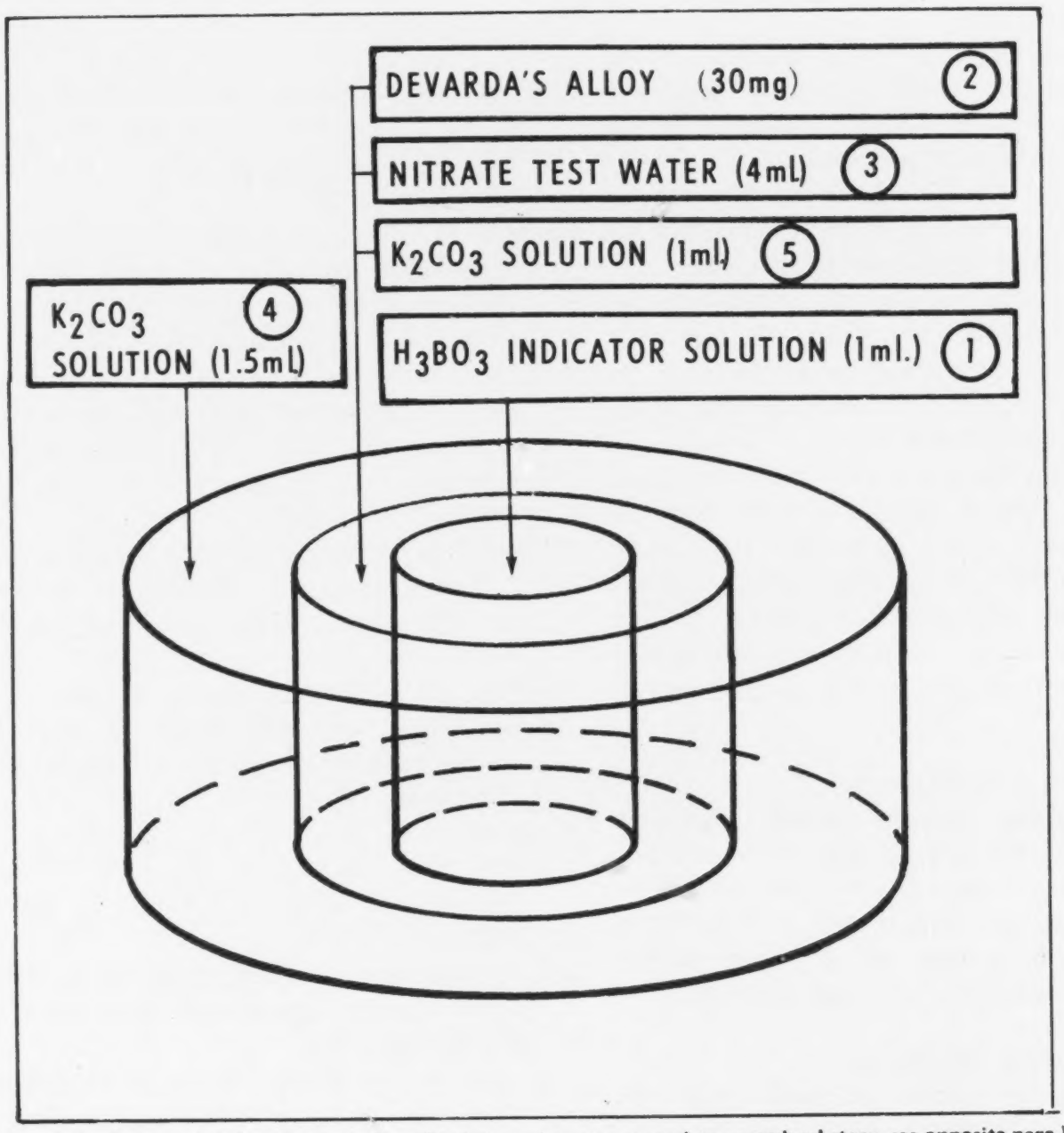


**Figure 5.—Conway dish for nitrate testing. (Circled numbers correspond to procedural steps, see opposite page.)**

4. Pipette 1.5 ml. of 40% $K_2CO_3$ solution into the outer ring.
5. Pipette 1 ml. of 40% $K_2CO_3$ solution into the ring containing the Devarda's alloy and the sample test water. The $K_2CO_3$ acts as a sealing agent.
6. Prepare the second Conway dish in the same way as the first dish, omitting the Devarda's alloy.
7. Prepare the third and fourth Conway dishes as controls in order to check on the purity of the reagents. Both should contain distilled water in place of the sample test water. One should contain Devarda's alloy; the other should not. All other contents are the same as in Dish #1.
8. Place the lids on the four dishes and allow them to stand overnight.

*Titration Procedure:*

9. After twenty-four hours, remove the lids and place a small amount of distilled water into the center chamber of all dishes to provide more volume.
10. Fill the microburette with .02 normal $H_2SO_4$ (sulfuric acid) and begin titrating the solution contained in the center ring. Record the number of microliters of $H_2SO_4$ needed to return the indicator back to its original color. (Be sure to rinse off the burette with distilled water before using it each time.)
11. Calculate the amount of nitrate nitrogen in the original sample as follows: Total acid used minus acid used by control times 280 = micrograms nitrate nitrogen (in Dish #1). Note: 1 mililiter of .02 normal $H_2SO_4$ will titrate 280 micrograms of nitrogen in the form of ammonium.

*Discussion of Control Procedure:*

If the water sample already contains ammonium, this must be determined without Devarda's alloy (see Step 6 of Procedure). In such a case, the titration from Dish #2 is subtracted from Dish #1 to determine the amount of nitrate-nitrogen (see Step 11).

*Suggested Uses for Techniques:*

1. Analysis of Water Nitrate (e.g., in pollution studies). According to U.S. Department of Health Standards, only 10 p.p.m. nitrate nitrogen is permissible in drinking water.
2. Analysis of Soil Nitrate (also important in pollution studies). Excess nitrate in the soil can be a contributing factor to water pollution as a result of soil runoff and downward leaching through the soil.
3. Analysis of Plant Nitrate—A study of the amounts of nitrates absorbed by a plant or used by plants during different periods of development.

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